NITROGEN MINERALIZATION OF SEVEN MULTIPURPOSE TREE GREEN MANURES: RATES, PATTERNS AND RESIDUAL EFFECTS ON SOIL N MINERALIZATION

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY
OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE
IN AGRONOMY AND SOIL SCIENCE

MAY 1990

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ACKNOWLEDGEMENTS

The support and encouragement I received during the past three years contributed to making my graduate work an enjoyable learning experience. I would first like to thank my major professor, Dr. James Fownes. Jim lent his support in the form of encouraging words, patience in teaching (and re-teaching) me how to calculate nitrogen mineralization rates, and sharing his enthusiasm about our project. I would also like to thank my colleague, Nancy Glover, who readily offered her insights and very valuable advice. I not only value the intellectual support I received from these people but the good times and friendship we shared.

I would like to thank the other members of my committee: Dr. Hue and Dr. Caldwell for their assistance and flexibility towards my schedule (as I prepared to move to Kauai). The folks in the Agricultural Diagnostic Service Center gave me a lot of their time and use of their materials during my data analysis. Thank you Randall Takaesu and Ernie Okazaki!

The farm crew at the Wailua Experiment Station in Kauai took especially good care of our experiment and data collection. Special thanks to Jimmy Oshita, Trinidad Raval and Christopher Bernabe. You were all fun to work with.

I owe a lot to Cheryl Palm whose thesis helped direct a lot of my work. Cheryl very generously sent me a copy of her thesis, reagents and methodology hints and took time out to discuss some of the issues I was coming up against. I highly value her suggestions and expertise.

There are so many others I would like to thank who helped me out in ways they might not be aware of: Randi Schneider, Jane Lewis, Jan Schultz, John Coats, Andreas Boehringer...Lastly, a big thanks to my family for cheering me on with phone calls, visits and their caring. And very lastly, but not least, I would like to thank John Wagner who has stood by me through "the whole thing" and helped me realize just how valuable a good friend can be.

ABSTRACT

Green manure management is believed to play an important role in the "success" of alleycropping practices. However, studies that look at this do no include twigs, involve artificial pre-treatments of the material and are usually limited to one or two popular multipurpose trees. The research described in these chapters had the following objectives: (1) compare the "litterbag" and the "buried bag" methods for estimating N release, (2) compare nitrogen mineralization rates from seven different green manures, (3) examine the relationship between green manure quality and cumulative N mineralized, (4) test whether the first-order kinetic model is adequate as an empirical predictor of N release from a mixture of leguminous leaves and twigs and (5) compare soil N mineralization when green manure residue is present and absent. Green manure nitrogen was mineralized in a complex pattern. Soil N mineralization rates were slower in soil with 11 and 19-week-old mulch residue than in soil where the residue had been removed.

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CHAPTER ONE

AN OVERVIEW

INTRODUCTION

Population growth, land-use pressures and rapid cultural changes have affected agricultural practices in several regions of the world. Intensified farming practices have often resulted in shorter crop rotations, erosion, and rapid depletion of soil nutrients. As these agricultural trends continue, farming practices that can be described as "multi-purpose", "low-input" and "sustainable" have increased in popularity. One such farming practice is alley cropping--the planting of crops between rows of trees. Alley cropping is "multi-purpose" in that it can provide a source of fuelwood, fodder, food, stakes and fertilizer. "Low-input" practices include substituting fertilizer with applications of cuttings from leguminous trees (green manures). "Sustainable" is an often-used term that has to do with maintaining or improving land production over long periods of time.

In order to help understand how and if alley cropping lives up to the terminology just described, there has been a lot of research measuring how much nitrogen is contributed by green manure. There are several considerations to take into account before using the data from such studies to help a farmer choose and manage a green manure and crop.

Firstly, nitrogen mineralization (the conversion of organic nitrogen to inorganic nitrogen) of green manures has been estimated with different methods that give different values for the same species. For example, one study used litterbags to estimate that Leucaena leaves mineralized high amounts of nitrogen in the first week (Wilson et al., 1986) whereas another study that used incubations of soil and Leucaena leaves found that Leucaena immobilized N in that same period (Palm, 1988).

Secondly, nitrogen mineralization studies differ in how the plant and soil material are prepared for incubation. Most green manure incubation studies involve artificial pre-treatment of the material such as drying, grinding, re-wetting, and constant moisture and temperature during incubation. These conditions are quite different from the field and will affect N mineralization (Stanford and Epstein, 1974; Myers et al., 1982; Birch 1958 and 1960; Thiagalingam and Kanehiro, 1980). Thirdly, nitrogen mineralization studies seldom include the twig component of green manure (Palm, 1988; Weeraratna, 1979 and 1982; Wilson et al., 1986; Yamoah et al., 1986; Budelman et al., 1985). In practice, twigs would most likely be included in a green manure application because of the labor involved in separating leaves and twigs. A fourth consideration is that only a limited number of leguminous tree green manures have been evaluated--primarily Leucaena and Gliricidia (Budelman et al., 1985; Wilson et al., 1986; Yamoah et al., 1986; Cornforth and Davis, 1968; Weeraratna 1979 and 1982). The vulnerability of *Leucaena* to *Heteropsylla cubana* Crawford, a sucking insect, suggests that it would be pragmatic to evaluate a larger number of species.

The experiments described in later chapters were designed with these four considerations in mind and therefore: involved careful consideration of the methods to be used to measure N mineralization; included twigs and leaves in all of the incubations; used fresh, intact plant material; and involved green manures from eight different leguminous tree species.

APPROACHES

There are several methods for estimating nitrogen mineralization of soil and plant material (Table 1.1). Although some studies use litterbags to measure N mineralization of leguminous material (Budelman et al., 1985; Yamoah et al., 1986; Palm et al., 1988), the litterbag method was not listed in Table 1.1 because it measures nitrogen decomposition and only indirectly estimates nitrogen mineralization. In choosing a method, it is important to consider who is going to use the nitrogen mineralization estimates and for what purpose. If data is being collected for farmer use it may be desirable to choose a method that is adapted to the field such as: litterbag,

TABLE 1.1 Comparison of methodologies used to measure N mineralization

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES
polyethylene bag [a]	soil placed in polyethylene bag and either incubated in lab or buried in field	temperature and aeration of soil in bag is similar to soil outside	bag easily punctured and data is quite variable
stoppered leaching tubes[b]	soil placed in tubes and mineral N is removed by leaching with 0.01M $CaCl_2$	can "factor out" inorganic N that is present at time 0	environment is very different from that in the field and soil is disturbed with sieving, drying, re-wetting, etc.
glass jar or stoppered conical flasks[c]	adjust soil moisture content and incubate in lab	because controlled environment, can study effects of select variables	
anaerobic incubation [d]	waterlog soil and measure accumulation of NH_4-N	relatively simple and can use shorter incubation periods	microbial processes are quite different from that in field
PVC tube[e]	open-ended PVC tube is pushed into soil and end up is covered with a plastic cup	soil core is relatively undisturbed	not simple and soil is poorly aerated

[1]Eno, 1969 [2]Stanford et al., 1974 [3]Vallis and Jones, 1973; Weeraratna 1979 and 1982
[4]Waring and Bremner (1964) as cited in Keeney, 1980 [5]Palm, 1988

polyethylene bag, and PVC tube methods (Table 1.1). Palm (1988) found that polyethylene bags incubated in the field gave highly variable data. The litterbag is simple and is thought to involve less data variability (Palm, 1988) but the nitrogen mineralization estimates can be inaccurate due to the indirect method of measurement. No studies were found that compared the nitrogen mineralization rate estimates derived with these different methods. Therefore, it is difficult to know which method will give the desired information. The second chapter describes a study that compared the nitrogen mineralization rate estimates derived for Cajanus cajan using the litterbag and the polyethylene bag (buried bag) methods.

There are numerous ways to express nitrogen release from green manures. N mineralization expressed as a rate or as % of initial N mineralized can provide both researcher and farmer with information that facilitates comparison of different green manures. Data expressed as % can then be applied to green manure production values for calculating the nitrogen contributed by a particular green manure in units of kg / ha. As mentioned earlier, methods for measuring nitrogen mineralization will result in different estimates so it is practical to either evaluate several leguminous species in one study or use a modeling approach.

A modeling approach involves finding a relationship between nitrogen mineralized and a characteristic of the material, describing this relationship with an equation, validating this relationship in additional studies and then using the relationship to predict nitrogen mineralization of material with known "characteristics". This approach can save a considerable amount of the time and effort involved in incubating plant material in nitrogen mineralization studies. Nitrogen mineralization has been found to have a strong relationship with: lignin and the ratio of lignin to nitrogen in forest litter (Aber and Melillo, 1982; Berg and McClaugherty et al., 1987); polyphenol concentrations in leguminous material (Vallis and Jones, 1973; Palm, 1988) and carbon to nitrogen ratios of leguminous material (Frankenberger and Abdelmagid, 1985). None of these studies included a mixture of leguminous leaves and twigs. Leguminous twigs can contain higher concentrations of polyphenols and lignins (Frankenberger and Abdelmagid, 1985) and this could affect how these qualitative properties of a leaf and twig mixture relate to N mineralization. One of the experiments described in Chapter 3 investigates the relationship between nitrogen mineralization and several quality characteristics of leguminous leaf and twig mixtures.

So far this discussion has focussed on nitrogen contributions from green manure application, however, several of the leguminous tree species used for green manure can also be used as fodder (Cheeke and Raharjo, 1987; Foster and Blight, 1983) which would entail removal of the material. How does removal or application of green manure affect soil nitrogen availability? Soil nitrogen mineralization rates can vary a great deal (Table 1.2). Therefore, green manure removal and application treatments should be done on the same soil at the same time. The last set of experiments described in this thesis studies the effect of removal and application of seven different types of green manure on soil nitrogen mineralization. The experiments were conducted over time which facilitated studying how green manure residue affects soil nitrogen mineralization rates.

The research described in this thesis evolved largely as a process of question and answer. Numerous "methods" studies raised questions with regard to the effect of twigs on N mineralization. Results from N mineralization studies done in the field suggested that there was a fairly wide range in the N mineralization rates of the seven green manures that had been used. A follow-up laboratory study was designed to investigate this further and to analyze which green manure properties were affecting the rate of mineralization. Lastly, the effect of removal and application of green manure on soil nitrogen mineralization was studied in order to obtain a better perspective of whether N from green manure contributes to alley cropping being described as "sustainable" and "low-input".

TABLE 1.2 Soil nitrogen mineralization rates of different soils

REFERENCE	STUDY CHARACTERISTICS	MEAN ANNUAL RAINFALL (mm)	MEAN ANNUAL TEMP. (°C)	SOIL %N	MG N MINERALIZED KG^{-1} SOIL WK^{-1}
Thiagalinagam and Kanehiro, 1973	Tropeptic Eutrorthox	1,200	22	0.17	2.83
	Humoxic Tropohumult	2,000	21	0.25	3.33
	Typic Chromustert	500	24	0.10	1.25
	Hydric Dystrandept	1,600	14	0.78	1.17
Vitousek and Denslow, 1986	mulched cacao plantation in Brazil	4,200	-	0.42	8.3-13.3
Palm, 1988	Typic Paleudult, Peru	2,200	26	0.11	
	mulched				1.61-2.45
	not mulched				1.33
Smith et al. 1977	fallow plots, Oklahoma	-	3-43		
	Ultisol			0.06	3.38
	Vertisol			0.15	1.38
	Entisol			0.12	6.50
Pastor et al. 1984	forest in Wisconsin	-	-	-	*
	Entisol				0.33
	Alfisol				0.50
	Spodosol				0.68-1.08
Carter and Rennie, 1982	zero and conventional tillage in Canada	approximately: 400	3	-	4.7-12.5
Study in Ch. 4, this thesis	alleycrop on an Oxisol	2,650	23	0.20	
	with residue				1.10-1.53
	no residue				0.97-1.61

* bulk density was assumed to be 1 g / cm^3

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CHAPTER TWO

A COMPARISON OF NITROGEN MINERALIZATION RATE ESTIMATES DERIVED FOR *CAJANUS CAJAN* USING THE LITTERBAG AND THE BURIED BAG METHODS

ABSTRACT

Caution must be exercised when using estimates of the nitrogen mineralization rate of green manure as a basis for choosing a green manure. Often these estimates are derived with different methods and these methods have not yet been compared. This study was designed to test whether the "litterbag" and the "buried bag" methods of estimating N mineralization gave different estimates of *Cajanus cajan*'s rate of N release. The study used two-month-old *Cajanus* that was incubated in the field for 0,9,19 and 30 days. These methods resulted in different estimates of the N mineralization rate between 9 and 30 days ($p < 0.05$). However, the cumulative N mineralized at 30 days appeared to be similar between methods.

INTRODUCTION

As people become aware of the value of long-term solutions, "sustainability", "nutrient cycling" and "low-input" have become popular terms in the agricultural field. Alleycropping--the planting of crops between rows of trees--is often promoted in connection with these adjectives as a viable farming practice for tropical areas (Plochberger, 1988; Wilson *et al.*, 1986). Alleycropping involves coordinating the management of several components such as trees, crop production and soil fertility. Improving the understanding of how these function individually and interactively will not only aid in understanding how nutrients cycle but will also improve the ability to make sound management decisions. In an attempt to better understand some of the interactive processes occurring in an alleycropped field, numerous studies have measured nitrogen mineralization of green manures (Palm, 1988; Wilson et al., 1986; Budelman et al., 1985). However, estimates of nitrogen mineralization (Nmin) of leguminous tree green manure can vary for the same tree species. For example, leaves from Cassia siamea have been reported to lose from 29% (Yamoah et al., 1986) to 58% (Wilson et al., 1986) of their initial nitrogen in eight weeks. Differences such as these make it difficult to choose and manage a green manure for nitrogen supply to crops.

There are several methods for estimating nitrogen mineralization of plant material. Some methods incubate leaf material and soil in glass containers and use inorganic N accumulated from the mixture of leaves and soil as an estimate of leaf N mineralization (Cornforth and Davis, 1968; Weeraratna 1979 and 1982). Because the soil Nmin values are not factored out before arriving at an estimate with this method, N mineralization values of a green manure could appear to be quite different when incubated with different soil types. Palm (1988) adapted the buried bag method (Eno, 1960; Westermann and Crothers, 1980; Smith et al., 1977) of measuring soil nitrogen mineralization. In this method, soil and leguminous leaves were placed in polyethylene bags and incubated either in the laboratory or in the field. By subtracting the inorganic N accumulated in a control, soil-only incubation from that accumulated in a soil + green manure incubation, Palm estimated Nmin of the leguminous material. This method has the disadvantage that moisture conditions remain constant and the advantage of providing close contact between the plant material and the soil while still allowing gaseous exchanges between the soil and the atmosphere (Westermann and Crothers, 1980; Gordon et al., 1987). Nitrogen decomposition measurements have been obtained using nylon mesh bags, "litterbags", that contain plant material and measuring the rate of nitrogen loss from the bag (Aranguren et al., 1982; Budelman et al., 1985; Wilson et al., 1986; Yamoah et al., 1986). Although it is a relatively simple method, it mistakingly assumes that all of the nitrogen lost from the bags has been mineralized. Therefore, material that crumbles through the mesh openings of the bag is measured as "N mineralized" even if it is immobilized.

Comparing Nmin methods between different studies can be misleading since there are differences between age and treatment of plant material and experimental conditions. In a litterbag study, Palm (1988) found that *Cajanus* had only mineralized approximately 22% of its nitrogen after eight weeks of incubation. In a separate study, using the buried bag method, Palm (1988) measured net immobilization of Cajanus N over an eight week period. Were these differences due to the measurement method or were they due to artifacts from the age of the material and its preparation? This experiment was designed to test the hypothesis that the litterbag and buried bag methods result in different estimates of nitrogen mineralization of Cajanus cajan. In addition, the effect of litterbag placement on nitrogen mineralized was examined.

MATERIALS AND METHODS

Eight-week-old Cajanus cajan leaves and twigs 1 cm or less in diameter were coarsely chopped and incubated for 9,19 and 30 days using the litterbag and buried bag methods. The trees had been growing on a Wailua clay at the University of Hawaii Experiment Station located in Waimanalo. The soil was classified as a very fine, kaolinitic, isohyperthermic Vertic Haplustoll (ph 6.1).

The treatments were:

1. Litterbags placed under tree canopies
2. Litterbags placed in the middle of the tree rows, approximately 1.25 meters away from the trees
3. Buried Bags

The treatments were located randomly throughout the field which measured 35m x 35m. All treatments were replicated five times each sampling period.

For the litterbag treatments, samples of 2 to 3.5 g (dry weight) Cajanus were placed in nylon, 0.75mm mesh bags that measured 40cm x 40cm. An "initial" sample of plant material was measured for dry weight and Kjeldahl N analysis (Isaac and Johnson, 1976; Schuman et al., 1973) and the other litterbags were distributed in the field. At each harvest interval, five bags from each litterbag treatment were randomly collected and dried at 70°C. Ground subsamples were analyzed for total Kjeldahl N and for percent ash. Weight loss was calculated on an ash-free dry weight basis to reduce the effects of adhering soil particles.

For the buried bag method, 15 pairs of soil samples were taken of a 0-25 cm depth and placed in polyethylene bags. Each pair was located randomly throughout the field. In addition, five more "initial" soil samples were taken and extracted the same day for inorganic N. Coarsely chopped Cajanus was added in a ratio of 0.0037 g plant material per gram of soil (dry weight basis) to one of each of the pairs of soil samples. All of the bags were knotted, returned to their sampling hole and covered with soil. Five pairs of bags were collected at each harvest interval. The control (soil-only) and soil+green manure samples were sieved through a 10 mesh sieve and a 15 g subsample was extracted for inorganic N in 100 ml of 2N KCl. After allowing the soil to settle overnight, a portion of the extract solution was pipetted, filtered and refrigerated until analysis one month later. Filtrates were analyzed for ammonium on a Technicon AutoAnalyzer II (Gentry and Willis, 1988) and for nitrate using ultraviolet spectrophotometry at 210 nm (Cawse, 1967).

Because data was expressed as percent of green manure nitrogen mineralized, it was necessary to take the arcsine of the data before comparing the nitrogen mineralization rate estimates derived from the buried bag and both the litterbag treatments. If the effect of treatment * time was significant ($p < 0.05$) in an analysis of regression, the rate estimates were considered to be different. "Time 0" data was not used in this statistical analysis since it was assumed that none of the *Cajanus* nitrogen had been mineralized at time 0.

RESULTS

The buried bag method gave a smaller estimate of the nitrogen mineralization rate of *Cajanus cajan* in the period of 9 to 30 days than either of the litterbag treatment estimates. At 30 days, the cumulative percent of green manure N mineralized was calculated to be 68% using the buried bag method; 74% using the litterbag placed in the open (LB,O); and 64% using the litterbag placed in the shade (LB,S) (Figure 2.1).

Litterbag placement did not affect the rate of N release from Cajanus for the period of 9 to 30 days in spite of 4°C changes in the daily ambient temperature as well as variable precipitation during the study (Figure 2.2),

DISCUSSION

The difference between the nitrogen mineralization estimates of the two methods from 9 to 30 days suggests that it is important to use the same measurement method to compare green manure species nitrogen mineralization rates. However, a more thorough test of the original hypothesis is needed before arriving at any conclusions. Experiments should be conducted with green manures having a range in quality. Including more frequent harvest intervals in a longer study would likely provide a more thorough comparison. Because litterbags really measure N loss and only indirectly give an estimate of N mineralization, it would be expected that the litterbag and buried bag methods would give increasingly different estimates of the nitrogen mineralization rate of Cajanus as the twigs decompose and possibly immobilize N.

The results from this study suggest that measurement methodologies result in different descriptions of the Nmin pattern of Cajanus (Figure 2.1). For example, the buried

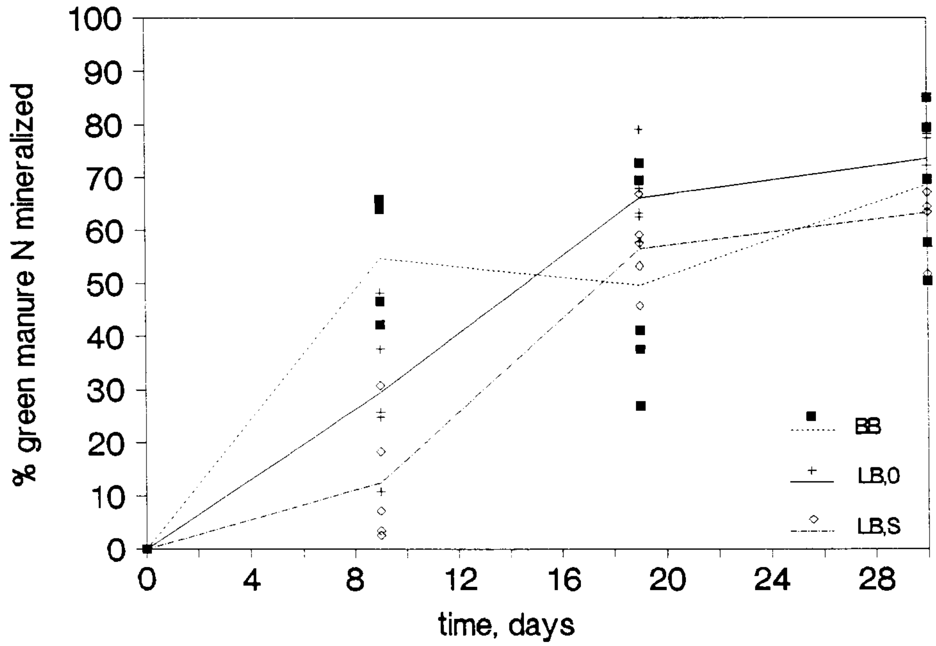

FIGURE 2.1 Cajanus cajan green manure nitrogen mineralization data derived from the buried bag (BB), litterbags placed in the open (LB,O) and litterbags placed in the shade (LB,S).

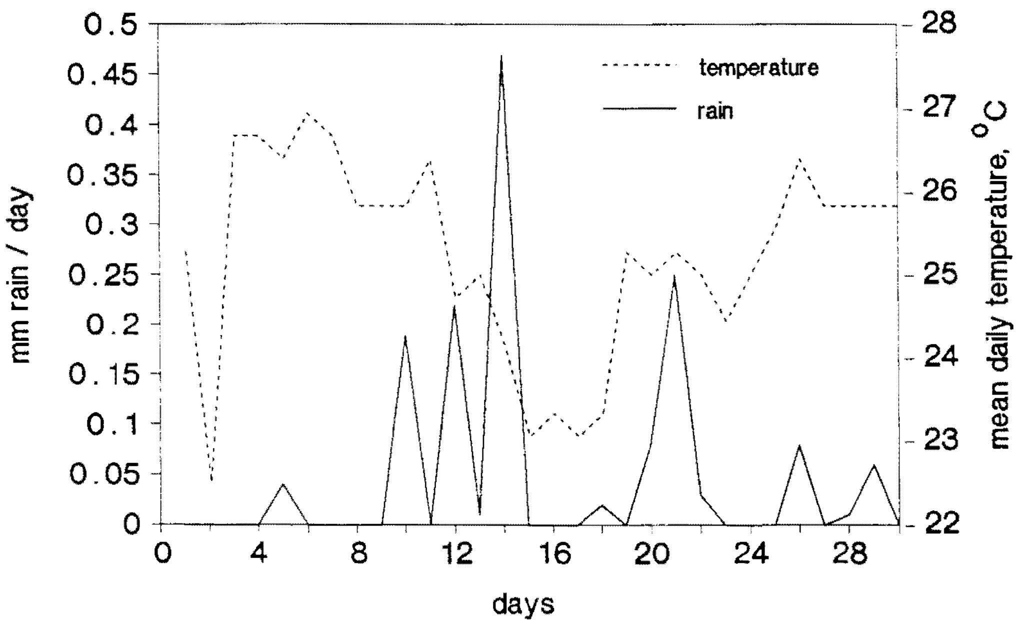

FIGURE 2.2 Daily temperature and rainfall occurring during the study period.

bag data suggests a decline in the Nmin rate of _Cajanus_ in the period of 9 to 19 days that is not indicated by data from either of the litterbag treatments. Variability of buried bag data, such as that shown at 9 days, has been found in soil-only buried bag studies (Palm, 1988; Matson and Vitousek, 1981; Burke, 1989) where coefficients of variation as high as 36% (Keeney, 1980) have been reported.

Several studies have shown that temperature and moisture can exert an important influence on the rate of nitrogen mineralization (Myers _et al._, 1982; Stanford and Epstein, 1974). The changes in precipitation and temperature during this study did not appear to have influenced the micro-climate conditions of the shade placement versus the in-the-open placement of the litterbags enough to effect different rates of nitrogen mineralization.

Not including the period of 0 to 9 days in the method comparison of the nitrogen mineralization rates of _Cajanus cajan_ ignores the apparent rapid rate of Nmin in the first 9 days that was measured with the buried bag method (Figure 2.1). Therefore, it is not possible to conclude that the buried bag results in lower Nmin estimates than the litterbag method. At 30 days, the percent of _Cajanus_ mineralized appears to be similar regardless of the method used to arrive at the estimate (Figure 2.1). This suggests that differences in the N mineralization estimates derived with the litterbag and buried bag methods may not be important if the green manure is being managed so that cumulative N mineralized at 30 days is more important than the rate of N release up to 30 days.

The rapid rate of Cajanus Nmin estimated by all of the methods in this study contrasts sharply with the slow Nmin rates calculated in both of Palm's (1988) Cajanus studies. This seems to indicate that other factors such as age of the material and pre-incubation treatment of the material could be effecting some of the observed differences in Nmin estimates for the same species. These indications along with the results from this study caution against comparing N release rates of green manures when they are measured with different methods or in different studies.

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CHAPTER 3
NITROGEN RELEASE FROM SEVEN GREEN MANURES AND THE EFFECT OF GREEN MANURE QUALITY ON CUMULATIVE N MINERALIZED

ABSTRACT

Estimates of leguminous green manure nitrogen mineralization exist for a limited number of species and rarely include twigs in the study. The first experiment described in this paper was a field study that compared N mineralization rates of leaf and twig green manure from seven tropical multipurpose trees. The second experiment used the same green manures in a laboratory study with the following objectives: (1) to test the first-order kinetic model as an empirical predictor of N release from complex substrates and (2) examine the relationship between green manure quality and the percent of added green manure N mineralized. Percent lignin, %N, %polyphenols, leaf/twig and C/N were determined for the initial plant material. The N mineralization patterns were complex and varied between species. In both experiments, *Gliricidia* had the highest rate of nitrogen mineralization and *Inga* had the lowest.

INTRODUCTION

Alleycropping practices are often advocated as a way to maintain or improve soil fertility over long periods of time (Kang *et al.*, 1986). One of the aims of alley cropping is to maximize efficient nutrient cycling and input while trying to minimize nutrient loss via leaching or runoff.

The quality of green manure applied in an alleycropping practice will affect these nutrient conserving processes which in turn can influence crop yields. A low quality green manure will decompose slowly and gradually contribute to build-up of the organic matter pool. Due to the ligneous nature of twigs, the twig component of green manure is an example of low quality material. The leaf component of leguminous material has higher concentrations of nitrogen than twigs (Frankenberger and Abdelmagid, 1985) and decomposes and mineralizes its nitrogen over relatively short periods of time (Frankenberger and Abdelmagid, 1985; Palm et al., 1988). Successful management of N release for crop N uptake or build-up of soil N requires knowing the rate of nitrogen release from the green manure. Studies that evaluate the N release of green manure deal almost exclusively with the leaf component and they are done with a limited number of species--either with Leucaena or with Gliricidia (Yamoah et al., 1986; Wilson et al., 1986; Weeraratna 1979 and 1982; Cornforth and Davis, 1968; Budelman, 1985). Different studies use different methodologies which result in different estimates of N mineralization (Chapter 2 of this thesis). Because of the additional labor required to remove twigs from green manure and because of the long-term benefits associated with low quality material (IITA, 1989; Palm, 1988), it is likely that twigs would be included in a green manure application. The preliminary study described in this paper was designed to compare N mineralization (Nmin) rates of leaf and twig mixtures of seven different green manures.

A second study was designed to: (1) examine the relationship between green manure quality and cumulative N mineralized of the seven green manures used in the preliminary study and (2) test whether the first-order kinetic model was adequate as an empirical predictor of N release from complex substrates. The first-order kinetic model:

$$\frac{N_t^{GM}}{N_o^{GM}} = e^{-kt}$$

N_t^{GM} = green manure N remaining at time t
= $(N_o^{GM} - N_{min}^{GM})$ where N_{min}^{GM} =cumulative Nmin from green manure at time t

N_o^{GM} = total N in green manure at time 0

k = N mineralization rate constant

has been used to describe decomposition (Aber and Melillo, 1980; Fogel and Cromack, 1977; Wieder and Lang, 1982) and N mineralization (Budelman <u>et al.</u>, 1985; Frankenberger and Abdelmagid, 1985; Palm, 1988) of plant material. However, studies that use this model to describe N mineralization do not use leaf and twig mixtures in incubations. Nitrogen mineralization of leguminous leaf and twig mixtures has not been studied. Because leguminous twigs have been found to be of lower quality than leguminous leaves (Frankenberger and Abdelmagid, 1985), it was hypothesized that the first-order kinetic model would not be adequate for describing N mineralization of a complex substrate such as that of a leguminous leaf and twig mixture.

A modeling approach can be used for predicting N mineralization by relating inorganic N accumulated from plant material to properties associated with quality and then use the equation that describes this relationship for predicting Nmin of other species. This has been attempted using the inverse relationship of temperate forest litter Nmin to lignin or the ratio of lignin to nitrogen of the material (Aber and Melillo, 1982; Berg and McClaugherty et al., 1985). The material used in these temperate studies was of low quality and had nitrogen contents that were usually below 1.5% and lignin concentrations that ranged from 10% to 25%. Only one published nitrogen mineralization study was found that included chemical quality analysis of leguminous twigs (Frankenberger and Abdelmagid, 1985). However, this study did not separate foliage, stem and root data when relating Nmin to qualitative properties. Frankenberger and Abdelmagid (1985) found a significant inverse relationship between cumulative nitrogen mineralized by leguminous material at 20 weeks and the lignin concentrations. Lignin contents ranged from 3.5% to 24.7% while nitrogen concentrations ranged from 1.34% to 5.88%.

Carbon to nitrogen ratios (C/N) have also been studied in conjunction with Nmin of both temperate forest litter

(Pastor et al., 1984) and leguminous material (Frankenberger and Abdelmagid, 1985; Weeraratna, 1979). However, using C/N ratios does not take into account differences in carbon quality. Also, leguminous green manures have C/N ratios that are usually not above 18 (Weeraratna, 1979; Frankenberger and Abdelmagid, 1985) which is at the low end of the 15-33 range usually cited for critical C/N values (Tisdale and Nelson, 1975; Frankenberger and Abdelmagid, 1985).

Studies with pasture legumes (Vallis and Jones, 1973) and leguminous tree leaves (Palm, 1988) have not found a strong relationship between nitrogen mineralization and lignin. However, there was a strong relationship between Nmin and polyphenol concentrations or the ratio of polyphenols to nitrogen. These studies used green leaf material that had lignin contents of 3.1 to 17.9%, 1.1 to 4.5% N and 0.69 to 4.5% water/ethanol soluble polyphenols.

Tannins are usually defined as water soluble polyphenolics that are associated with protein but are not linked with cell wall carbohydrates while lignins are defined as polymerized phenyl propanoid units covalently linked to cell wall carbohydrates and not extractable with acid (Wong, 1973; Van Soest and Robertson, 1985). However, the extraction methods for tannins can confuse the distinction between lignin and tannins. The Folin-Denis colorimetric method reacts with hydrolyzable tannins along with condensed tannins and non-tannin polyphenols. Tannins plus other polyphenolic substances as extracted by a water/ethanol mixture will be referred to as polyphenols in this paper.

Since it was hypothesized that the combination of leaves and twigs would result in complex patterns of nitrogen mineralization, a method that is both able to measure Nmin directly and can detect immobilization should be used to measure Nmin. For this reason (see Chapter 2 of this thesis), the buried bag method was chosen for measuring N mineralization.

MATERIALS AND METHODS

DESCRIPTION OF EXPERIMENTAL MATERIAL

Nitrogen mineralization was measured by incubating green manure and soil in polyethylene bags (Palm, 1988). Green manure consisted of leaves and twigs (twigs 1 cm or less in diameter). Leaves and twigs were chopped so that leaves were less than 2 cm^2 and twig cross sections had diameters less than 0.3 cm. The green manure was taken from an alleycropping trial conducted at the University of Hawaii Wailua Experiment Station on the island of Kauai. Green manures from the following tree species were used:

Calliandra calothyrsus
Cassia reticulata
Cassia siamea
Gliricidia sepium (Oxford Forestry Institute collected in Guatemala: CSI14-85, referred to in this text as Gliricidia 1)
Gliricidia sepium (bulk composite of four selected provenances, referred to in this text as Gliricidia 2)
Inga edulis
Leucaena leucocephala (K636)
Sesbania sesban

The green manure material for the first Nmin study came from trees that were seven to nine months old. The plant material used in the second study came from trees that were 14-16 months old. The soil at the Wailua site is a gravelly silty clay classified as a clayey, ferritic isothermic Typic Gibbsihumox of the Kapaa series (pH 4.5; 3.7% organic carbon; 0.2% total N).

PRELIMINARY STUDY COMPARING NMIN RATES OF GREEN MANURES

This was a field study arranged as an Augmented Randomized Complete Block Design with two replications of Leucaena, Inga, Cassia reticulata and Gliricidia 2 and four replications of the other green manures. Sixteen soil cores of depths representing 0-25 cm were taken in each replication of each species. To eight of the 16 soil bags, 0.005 to 0.01 g of a leaf and twig mixture was added per gram of soil (dry weight basis). Four of the green manure mixture bags and four of the soil-only bags were returned to their original samples holes, covered with soil and left to incubate. The remaining eight bags were returned to the lab. The contents of each sample were sieved through a 10 mesh sieve and a 15 g subsample was extracted for inorganic N in 100 ml of 2N KCl. After allowing the soil to settle overnight, a portion of the extract solution was pipetted, filtered and frozen until later analysis. At 4.5 and 8 weeks, half of the soil + green manure bags and half of the soil-only bags were removed from the field. These were extracted for inorganic N in the same manner as the time zero samples. Defrosted filtrates were analyzed on a Technicon AutoAnalyzer II for $NH4^+$ (Gentry and Willis, 1988), $NO3^+$ and $NO2^-$ (Technicon, 1977).

To facilitate possible later application of the data, inorganic N values for green manure N mineralization were expressed as % green manure nitrogen mineralized. The steps in arriving at this expression are shown below:

1. $N_{min}^{GM+soil} = (N_t^{inorg} - N_o^{inorg})$ = cumulative Nmin at time t

   N_o^{inorg} = inorganic N in soil and green manure mixture at time 0

   N_t^{inorg} = inorganic N in soil and green manure mixture at a later time, t

2. $N_{min}^{GM} = N_{min}^{GM+soil} - N_{min}^{soil}$ = cumulative Nmin from green manure at time t

   N_{min}^{soil} = cumulative Nmin of soil-only at time t

3. $\%N^{GM} = (N_{min}^{GM} / N_o^{GM}) * 100$ = cumulative % of initial green manure nitrogen mineralized at time, t

   N_o^{GM} = total N of green manure at time 0 per unit weight of soil

The nitrogen mineralization rates of the green manures were compared using analysis of regression (Table 3.2) and a significance level of 0.05. Because data was expressed as a percent, it was necessary to take the arcsine of the values before using analysis of regression. "Time 0" data was not used in this statistical analysis since it was assumed that 0% nitrogen had been mineralized at time 0.

FOLLOW-UP GREEN MANURE NMIN STUDY

Experimental Design

This laboratory study used a Randomized Complete Block Design with six replications of each green manure species and seven replications of the control (soil-only) at each time period. Gliricidia 2 was not used in this study. The soil used in the incubations was first sieved at field moisture through a 10 mesh sieve and then 45-65 g samples were placed in polyethylene bags. Between 0.002 and 0.004 g of green manure were added to each gram of soil (dry weight basis). The ratio of leaves to twigs added was the same as found in a green manure harvest of these trees six months earlier. Ten of the control, soil-only bags were extracted for inorganic N at "time 0" using the same procedure described earlier for the preliminary study. The remainder of the bags were incubated for 1, 2.5, 4, 8 and 12 weeks at 24°C. At each incubation period, soil and soil + green manure were extracted for inorganic N. Defrosted filtrates were analyzed using the same methods described for the preliminary study.

Testing the Adequacy of the First-Order Kinetic Model

The use of the first-order kinetic model as an empirical predictor of N release from complex substrates was tested using analysis of regression on data expressed as the natural log of the fraction of green manure nitrogen remaining:

$$\ln\ (N_t^{GM}\ /\ N_o^{GM})$$

(using the definitions described in the preliminary study)

The effect of time was broken down into its various components for the analysis of regression (Table 3.3). In this way, the trends of N mineralization could be studied in more depth.

Determination of Chemical Composition of Green Manure

Green manure with the same leaf to twig ratios as that added to the bags was analyzed for initial contents of: nitrogen, lignin and organic carbon. Plant material to be analyzed was dried at 55°C and ground to $\leq$ 1mm in a Wiley Mill. Total nitrogen was determined by Kjeldahl digestion followed by measurement using an indophenol blue method (Isaac and Johnson, 1976; Schuman et al., 1973). Lignin was determined by the acid detergent fiber method (USDA, 1970) and organic carbon was determined by a modified Walkley-Black method (Heanes, 1984).

Separate determinations for polyphenol content were done on dried and ground leaves and twigs (0.75 g) that were extracted in 20 ml of 50% aqueous methanol, heated to 77°C, filtered (Palm, 1988) and analyzed for tannins using the Folin-Denis reagent (sodium tungstate, phosphomolybdic acid and ortho-phosphoric acid) as described in Allen et al., 1974). Fifty-percent aqueous methanol soluble polyphenols are referred to simply as "polyphenols" in later sections of this paper.

Single and multiple linear regressions were done to study the relationship between N mineralized at specific time periods and the quality of the initial plant material. The statistical significance of these regressions could not be reported because these regressions were done on data expressed as percent of green manure nitrogen mineralized and not on the arcsine of this percent. Nevertheless, R^2 values were reported to give an approximation of how the strength of the relationships compared for different green manure properties.

RESULTS

PRELIMINARY STUDY

The nitrogen mineralization rates were similar for all of the green manures used in the preliminary, field study. *Gliricidia* 1 mineralized 66% of its nitrogen in eight weeks whereas *Inga* mineralized 12% in the same period (Figure 3.1).

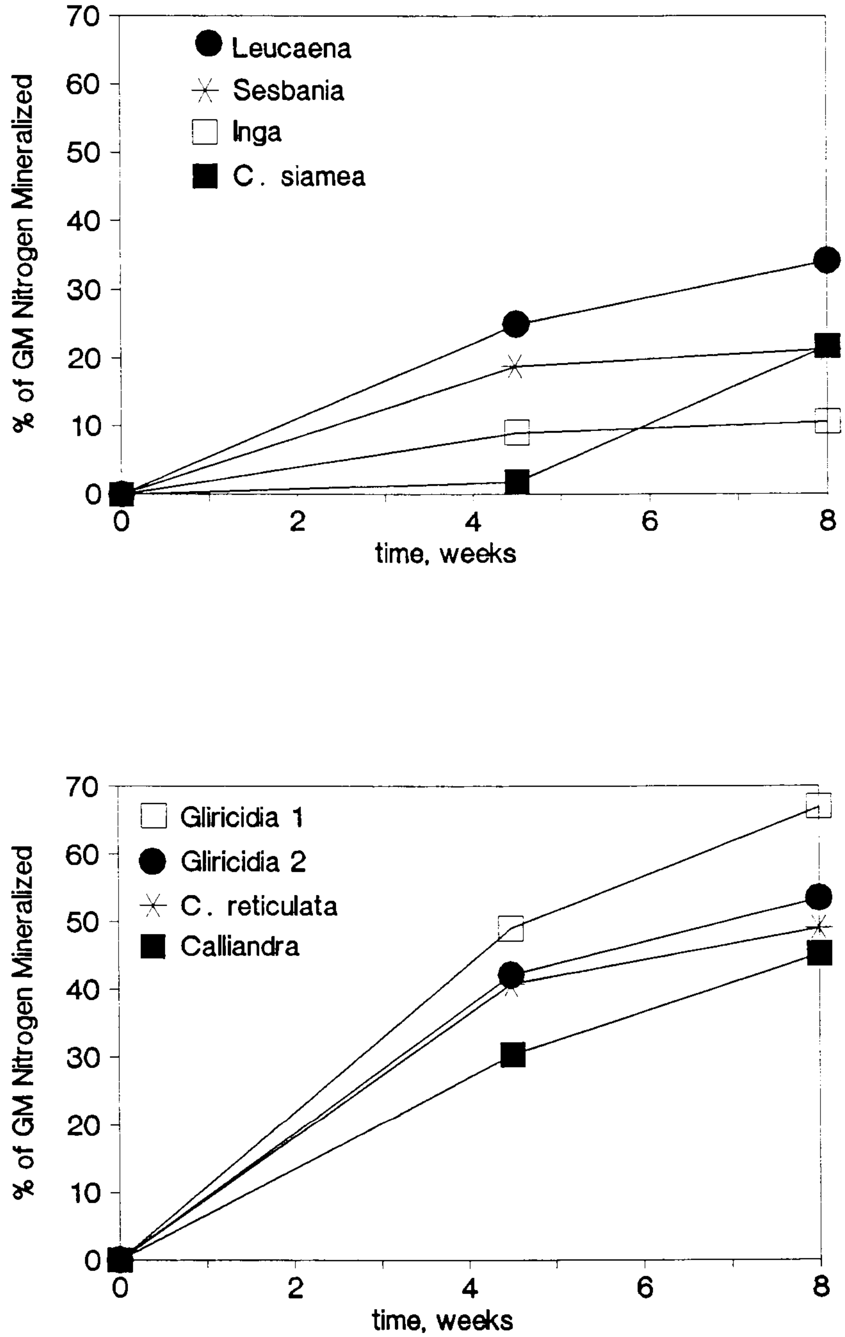

FIGURE 3.1 Nitrogen mineralization of different green manures used in the preliminary study. Values are the average of 8-16 samples (time 0) and 4-8 samples (4.5 to 8 weeks).

FOLLOW-UP GREEN MANURE NMIN STUDY

Testing the Adequacy of the First-Order Kinetic Model

The first-order kinetic model did not account for the variation among treatments. The actual pattern of mineralization followed a high order polynomial (Figure 3.2 and Table 3.3). Coefficients for the polynomial differed among species.

The Relationship Between Green Manure Properties and Nmin

There was a strong negative relationship between the % green manure nitrogen mineralized at 1, 2.5, 4 and 8 weeks and the ratio of %polyphenolics/ %N of the green manure (Figure 3.3). *Sesbania*, *Cassia siamea* and *Calliandra* had the highest polyphenol to nitrogen ratios whereas *Gliricidia* had the lowest ratio. The regression equations relating Nmin to the ratio of %polyphenolics to %N were similar for the 2.5, 4 and 8 week periods. With Y representing the percent of green manure nitrogen mineralized and X representing the polyphenol/N ratio, this relationship was best described at 2.5 weeks with the equation:

$$Y = -39.18X + 66.23 \quad (R^2 = 0.88)$$

Percent nitrogen of the green manure ranged from a low of 1.39% for *Sesbania* to a high of 3.74% for *Leucaena* (Table 3.1). Carbon to nitrogen ratios ranged from 12.70 for

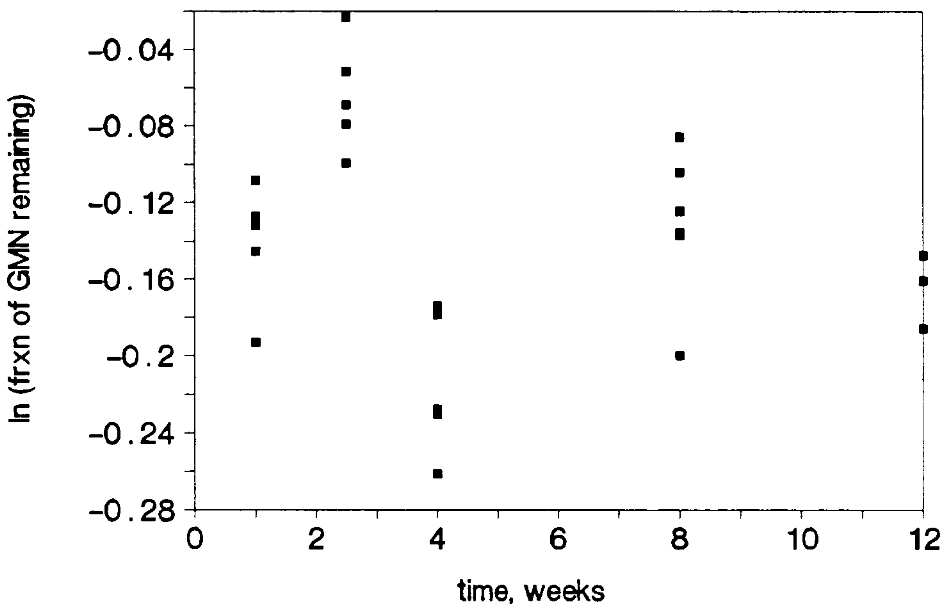

FIGURE 3.2 An example of the follow-up study data which illustrates the complex patterns of nitrogen mineralization of leaf and twig green manure.

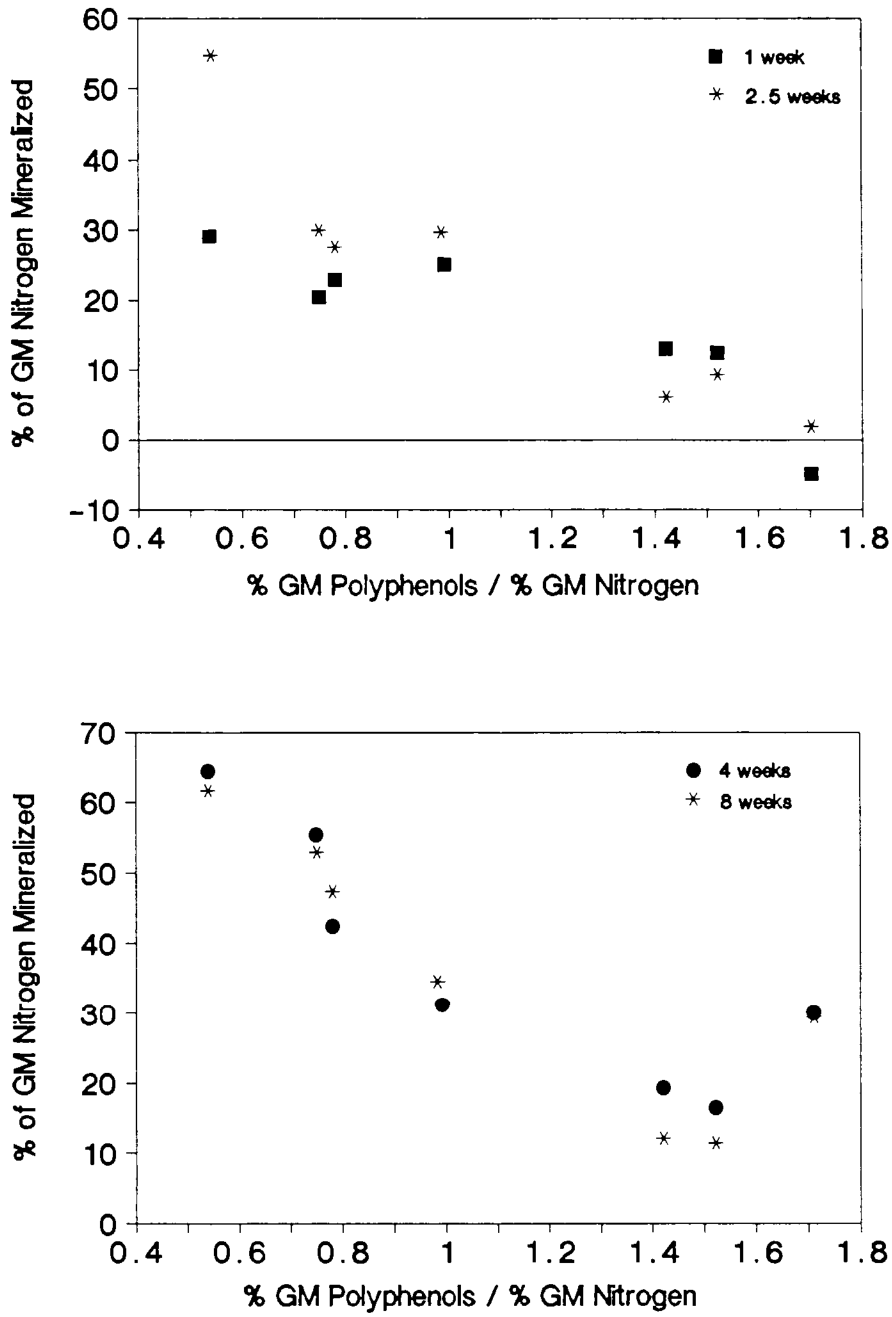

FIGURE 3.3 Percent of green manure (GM) nitrogen mineralized at various time periods in relation to the ratio of %polyphenols to %N. Individual points represent mean values for each species.

TABLE 3.1 Green manure properties.

SPECIES	GREEN MANURE PROPERTIES (DRY WT. BASIS)								
	POLYPHENOLS								
	% LEAF	% TWIG	TOTAL	%DRY MATTER	% LIGNIN	% NITROGEN	C/N	LEAF/TWIG	POLYPHENOL/ NITROGEN
Leucaena leucocephala	3.52	1.49	2.93	30.2	11.1	3.74	12.70	2.4	0.78
Inga edulis	4.71	1.70	3.83	31.0	18.3	2.51	18.96	2.4	1.52
Sesbania sesban	2.60	0.56	1.38	32.8	14.5	1.39	34.16	0.7	0.99
Cassia siamea	4.60	1.27	3.92	27.3	10.3	2.31	20.09	3.9	1.70
Calliandra calothyrsus	4.99	1.95	4.06	33.7	13.4	2.85	16.96	2.3	1.42
Gliricidia sepium	2.07	0.35	1.84	18.1	8.6	3.43	13.84	6.4	0.54
Cassia reticulata	2.10	0.89	1.99	23.6	9.9	2.65	17.68	9.4	0.75

Leucaena to 34.16 for Sesbania (Table 3.1). The leaf to twig ratios ranged from a low of 0.7 for Sesbania to a high of 9.4 for Cassia reticulata. There were weak relationships between the values of these green manure properties and the percent of green manure nitrogen mineralized at any of the time periods (R^2 values were less than 0.27 for single regressions).

The percent lignin of the green manure ranged from 8.6 for Gliricidia to 18.3 for Inga (Table 3.1). The percent of green manure N mineralized was inversely related to lignin concentrations with stronger relationships existing towards 4,8 and 12 weeks (R^2 <0.64). The total polyphenolic concentration of the green manure ranged from 1.38% for Sesbania to 4.06% for Calliandra (Table 3.1). Percent green manure nitrogen mineralized was inversely related to this chemical characteristic and was strongest at 2.5 weeks (R2 = 0.73). Multiple regressions of % green manure N mineralized at 4 and 8 weeks against total polyphenolics + green manure nitrogen or total polyphenolics +C/N were some of the few multiple regressions that had R^2 values as high as 0.91.

The only green manure properties with strong relationships to nitrogen mineralized at 12 weeks were twig polyphenols, %lignin and %dry matter of the plant material (R^2 = 0.70, 0.62 and 0.56 respectively). Each of these characteristics was negatively related to %green manure N mineralized at 12 weeks. The equation relating Nmin at 12 weeks (Y) with twig polyphenol concentrations (X) was:

$$Y = -26.66x + 66.68$$

DISCUSSION

GREEN MANURE N MINERALIZATION RATES

Except for Calliandra green manure, a high to low ranking of the average rates of nitrogen mineralization by species was similar between the preliminary study and the follow-up study. The high variability (coefficients of variation of approximately 58% at each time period) of the field incubations was one of the incentives to do a follow-up study under laboratory conditions. Water leaked into 12% of the bags in the field study making the liquid contents un-extractable. Punctures appeared to be caused either by insects (ants were found inside some bags) or by scratches and tears from gravel or twig material.

Both studies found that Cassia reticulata, Leucaena and Gliricidia were relatively fast N releasers when compared to the other species used in the incubations (Figure 3.1 and 3.4). Gliricidia leaf nitrogen has been shown to be quickly mineralized (Palm, 1988; Budelman et al., 1985; Cornforth and Davis, 1968). Several studies have found that Leucaena N mineralized two to four times faster (Weeraratna, 1982; Wilson et al., 1986) than was found in either the preliminary or follow-up studies. Palm et al., 1988 found

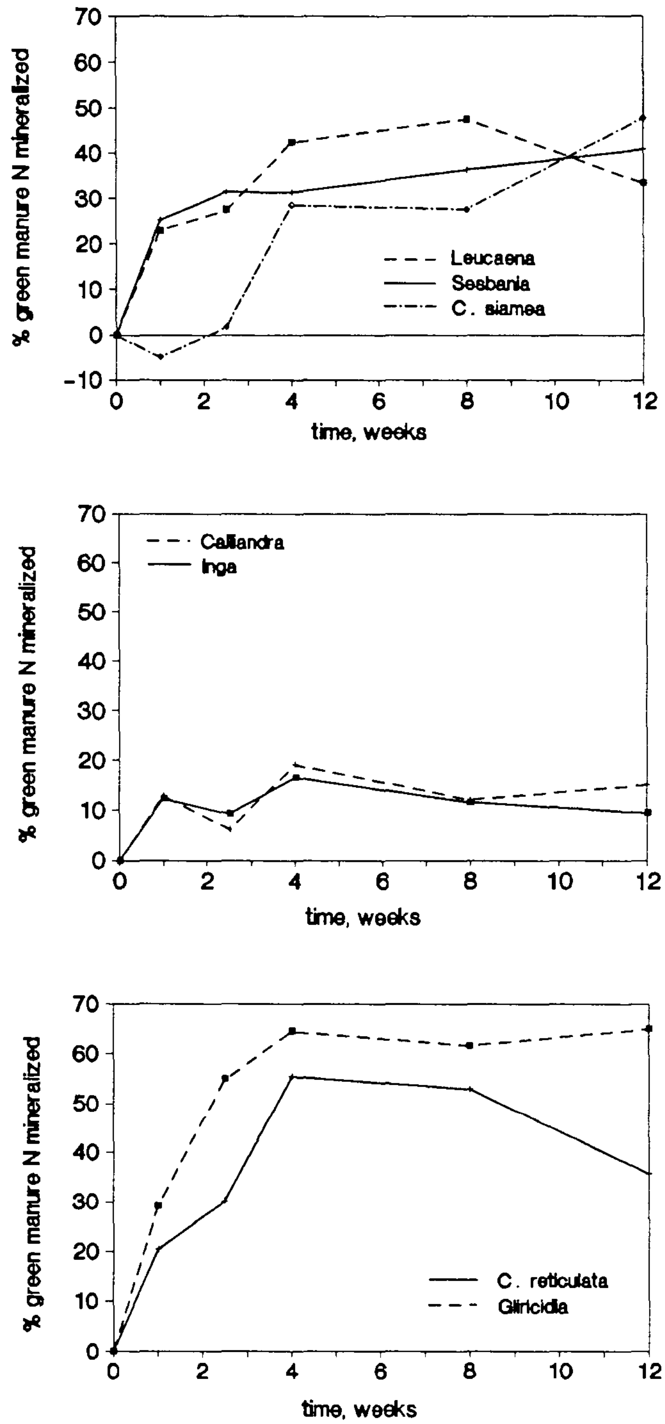

FIGURE 3.4 Nitrogen mineralization of green manures used in the follow-up incubation study. Values are the average of six replications.

that Sesbania leaves mineralized most of the nitrogen within 2.5 weeks. Slower rates of nitrogen mineralization would be expected when twigs are included in the incubation, as they were in this study. Examination of the contents of the bags showed that twigs were beginning to decompose as early as 2.5 weeks, thus possibly explaining the slower Nmin rates found in this study.

The slow release of Inga's nitrogen that was found in both the preliminary and follow-up studies could be considered a desirable attribute. IITA (1989) found that the residual effects of a green manure mixture of Leucaena and Cassia siamea resulted in yields that were 45% higher than crops that had received only Leucaena green manure in the previous season. It appears that Leucaena and Cassia reticulata might have immobilized nitrogen in the period of 8 to 12 weeks (Figure 3.4). It is not possible to compare these results to other published studies since nitrogen release has not been evaluated beyond 8 weeks for these particular species. Leaching and volatilization of nitrogen could potentially be reduced by coordinating N immobilization of low quality materials with fast N release from high quality materials. This type of management would likely contribute to both short and long term effects on soil N and crop yields.

TESTING THE ADEQUACY OF THE FIRST-ORDER KINETIC MODEL

The patterns of nitrogen mineralization found in the follow-up study were more complex than those found in studies that incubated leguminous leaves and twigs separately (Yamoah et al., 1986; Weeraratna, 1979; Palm et al., 1988; Frankenberger and Abdelmagid, 1985). This, together with observations of twig decomposition as early as 2.5 weeks for some of the species, strongly suggests that twigs play a role in affecting the degree of complexity of the Nmin patterns. Therefore, including twigs in a determination of green manure nitrogen mineralization results in a different description of the pattern of nitrogen release. For example, the decline in the Nmin rate indicated by Cassia siamea in the early part of the incubation (Figure 3.4) was not found in studies that incubated only leaf material from this species (Yamoah et al., 1986; Wilson et al., 1986). In fact, Wilson et al. (1986) found that Cassia siamea leaf N decomposed quickly in the first week followed by a high rate of N mineralization. Further experimentation involving separate incubations of leaves and twigs would be necessary to examine if and when twigs are affecting fluctuations in Nmin rates. It would be especially interesting to focus on time periods when leaves, or "fast N pools" are still decomposing and when twigs, or "slow N pools" are also decomposing.

THE RELATIONSHIP BETWEEN GREEN MANURE PROPERTIES AND NMIN

The relationships between nitrogen mineralized and various green manure properties suggest that the ratio of percent polyphenols to initial N concentration of the plant material has potential for predicting cumulative N released by leguminous leaf and twig material up to eight weeks of incubation. The concentration of polyphenols in twigs has potential for predicting N mineralized at 12 weeks. Because the green manures used in this experiment represented a range in values for each property, it is possible that these relationships could be used for predicting N release from a variety of green manure types. Further experimentation would be necessary in order to test whether the regression equations derived in this study would predict N release of other green manures in other studies. Factors such as leaf/twig ratios and age of the plant material may be important in determining how valid these relationships are outside of this study. However, the lack of a relationship between leaf/twig ratios and Nmin in this study and the precedence in other studies (Palm, 1988; Vallis and Jones, 1973) for finding the same lack of relationship suggests that model-approach research would be worthwhile.

Defining which polyphenols are reducing N mineralization would likely improve the precision of prediction. Using two herbaceous legumes , Vallis and Jones (1973) concluded that a small proportion of the total polyphenols were responsible for affecting N release. Defining specific polyphenols can be a difficult task when considering that different extraction methods have different efficiencies of extraction (Swain, 1979) and do not necessary extract the same types of polyphenols (Van Soest and Robertson, 1985). There can also be differences in the quality of the same species of material. For example, although the same method of polyphenolic determination was used in this study as in Palm's study (1988), the concentration of leaf polyphenols for three of the four species used in each study were much higher (16-50% higher) in this study. These differences could be affected by age of the tree, environment, variety of the species or even possibly by Palm's drying of the leaf material.

CONCLUSIONS

This study found a large range in green manure quality. High quality green manures mineralized as much as 68% of their nitrogen within a 12 week period whereas low quality green manures mineralized as little as 10%. Visual observations of the leaf and twig material at 12 weeks indicated that although by 12 weeks twigs of all the species showed signs of decomposition, they were all still relatively intact. This suggests that even the high quality green manures may have some long-term residual effects on available soil N supplies. Because the pattern of nitrogen mineralization of a complex substrate such as leaf and twig mixtures could not be described with the first-order kinetic model, it is difficult to predict when N immobilization or mineralization would be influencing the nature of these long-term effects.

The ratio of green manure polyphenol/nitrogen has potential for predicting the percentage of N released by different species of green manures up to eight weeks after application. At 12 weeks, nitrogen mineralized appears to be more related to twig polyphenol concentrations than any other green manure properties studied. The ability to select and manage a species of green manure could be improved if these relationships are validated in later studies. In selecting a green manure it is also important to keep in mind the "multi-purpose" uses of green manure such as erosion and weed control, livestock feed, moisture conservation, and the contribution of other important nutrients such as phosphorus and pottasium.

TABLE 3.2. ANOVA for the preliminary study comparing the arcsine of the percent of nitrogen mineralized by the different green manures (species x time interaction was used to compare rates).

Source of Variation	df
replication	3
species	7
replication x species	13
time	1
time x replication	3
species x time	7

TABLE 3.3. ANOVA for follow-up study testing whether the first-order kinetic model could be used as an empirical predictor of N release from leaf and twig mixtures.

Source of Variation	df	Sums of Squares	Mean Squares	Sig.
species	6	9.64	1.61	**
time, linear	1	1.78	1.78	**
time^2 (quadratic)	1	0.90	0.90	**
time^3 (cubic)	1	0.19	0.19	**
time^4 (quartic)	1	0.25	0.25	**
species*time	6	1.14	0.19	**
species*time^2	6	1.04	0.17	**
species*time^3	6	0.35	0.06	**
species*time^4	6	0.20	0.03	*
error	149	1.94	0.01	

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CHAPTER 4

THE RESIDUAL EFFECTS OF GREEN MANURE REMOVAL AND APPLICATION ON SOIL NITROGEN MINERALIZATION

ABSTRACT

Although there are several studies that measure nitrogen release from leguminous leaf green manure, there is very little research that examines the effect of green manure residue on soil nitrogen mineralization. This study was designed to test the hypothesis that soil with leaf and twig residue would have higher nitrogen mineralization rates than soil with no residue. The study consisted of three experiments conducted separately over six months. Residue from seven different green manure was used: *Leucaena leucocephala*, *Gliricidia sepium*, *Calliandra calothyrsus*, *Sesbania sesban*, *Inga edulis*, *Cassia siamea* and *Cassia reticulata*. The effect of different green manure residues on soil nitrogen mineralization was also examined.

INTRODUCTION

Most studies that measure nitrogen release from green manure do not include twigs in the incubations (Palm, 1988; Yamoah *et al.*, 1986; Cornforth and Davis, 1968; Budelman *et al.*, 1985). In addition, leaves are usually dried, ground and incubated under constant temperature with sieved soil. In practice, twigs are included in the green manure application for such reasons as their residual contribution to soil organic matter as well as for saving labor cost from separating leaves and twigs. Also, the micro-environment created by moist, intact leaves incubating with a heterogenous soil mixture under fluctuating temperature conditions, is likely to affect nitrogen mineralization (Nmin) rates differently than the homogeneous conditions found in the laboratories (Thiagalingam and Kanehiro, 1980; Vitousek and Matson, 1985; Keeney, 1980). There are very few studies of nitrogen mineralization of leguminous twigs and no studies were found that measured nitrogen mineralization of leguminous leaf and twig mixtures or their effect on soil nitrogen mineralization. Although, leguminous twigs have been found to mineralize N more slowly than leaves (Frankenberger and Abdelmagid, 1985; Palm et al., 1988), it is not possible to know from this information alone how much nitrogen will be released--or immobilized--by a leaf and twig mixture.

Applying a leaf and twig mixture to a field will have residual effects on the soil which can influence crop production. Frankenberger and Abdelmagid (1985) found that cowpea and soybean stems exhibited net immobilization of nitrogen over a 20 week period whereas the leaves mineralized 36% and 76% of their nitrogen in this time period. Low quality temperate forest litter has been shown to immobilize nitrogen for up to two years (McClaugherty et al., 1985). These long-term residual effects can be favorable. Palm (1988) found that soil receiving low quality leaf material mineralized at a faster rate after 1.5 years than soil that had received higher quality mulch. IITA (1989) found that after two cropping seasons, maize yields were higher for treatments receiving low quality leguminous leaves than treatments receiving high quality material.

It may also be a management option to remove green manure for such uses as fodder, erosion control or mulching elsewhere. However, few studies discuss the impact of this removal on soil nitrogen mineralization. Every 17 weeks, Palm (1988) examined soil Nmin with and without leaf mulch application. Palm found that, in general, more nitrogen was mineralized in the mulched plots versus in the no mulch plots. It would be expected that green manure removal would have residual effects on the soil also since trees are using nitrogen to produce the green manure material which is then removed from the system.

Leguminous tree residue from seven species of trees was used in this study. The leaf material from all of these species has been used as fodder and therefore the green manure from these trees could be removed for livestock feed. One of the objective of the study was to examine the residual effects of the different types of green manure on soil nitrogen mineralization rates. It was hypothesized that nitrogen mineralization rates would be greater in soil that had mulch residue than in soil that did not have mulch residue.

METHODS

DESCRIPTION OF THE EXPERIMENTAL DESIGN AND SITE

Soil nitrogen mineralization was measured in an alley-cropping experiment conducted at the University of Hawaii Wailua Experiment Station located on the island of Kauai. The soil is a gravelly silty clay classified as a clayey, ferritic isothermic Typic Gibbsihumox, Kapaa series (pH 4.5; 3.7% organic carbon; 0.02% total N)

Seedlings from the following list of tree species were planted on March 22, 1988.

- *Calliandra calothyrsus*
- *Cassia reticulata*
- *Cassia saimea*
- *Gliricidia sepium* (Oxford Forestry Institute collected in Guatemala: CSI14-85. Referred to in this text as *Gliricidia* 1)
- *Gliricidia sepium* (bulk composite of four selected provenances. Referred to in this text as *Gliricidia* 2)
- *Inga edulis*
- *Leucaena leucocephala* (K636)
- *Sesbania sesban*

Plots were arranged in an augmented randomized complete block design, including a control with no tree and no residue (referred to as "no tree").

The trees were pruned five months after transplanting to the field. The green manure residue treatment consisted of incorporating the green manure leaves and twigs (twigs were defined as stems with a diameter of 1 cm or less) within the plot. The no green manure residue treatment consisted of removing all of the green manure from the field. Calliandra calothyrsus, Cassia siamea, Sesbania sesban and Gliricidia 1 received both the with and without residue treatments with four replications. Cassia reticulata, Inga edulis, Leucaena leucocephala and Gliricidia 2 received only the "with residue" treatment and had two replications. Field corn was planted two weeks after the experiment had begun. Ten days before corn tasselling, the trees were again pruned and the green manure treatments were repeated. The only difference was that the green manure for the mulch treatment was applied to the surface and not incorporated.

SOIL NITROGEN MINERALIZATION MEASUREMENTS

Three separate soil nitrogen mineralization experiments were sequentially conducted (Figure 4.1). The first experiment began one day prior to when the green manure was first applied. Therefore, this experiment did not include a mulch residue treatment. The with-residue treatment in the second experiment consisted of soil and whatever green manure residue remained from the first coppicing. The third experiment began 11 weeks after the second pruning of the trees and therefore soil samples for the with-residue treatment included residue from both the first and the second green manure application.

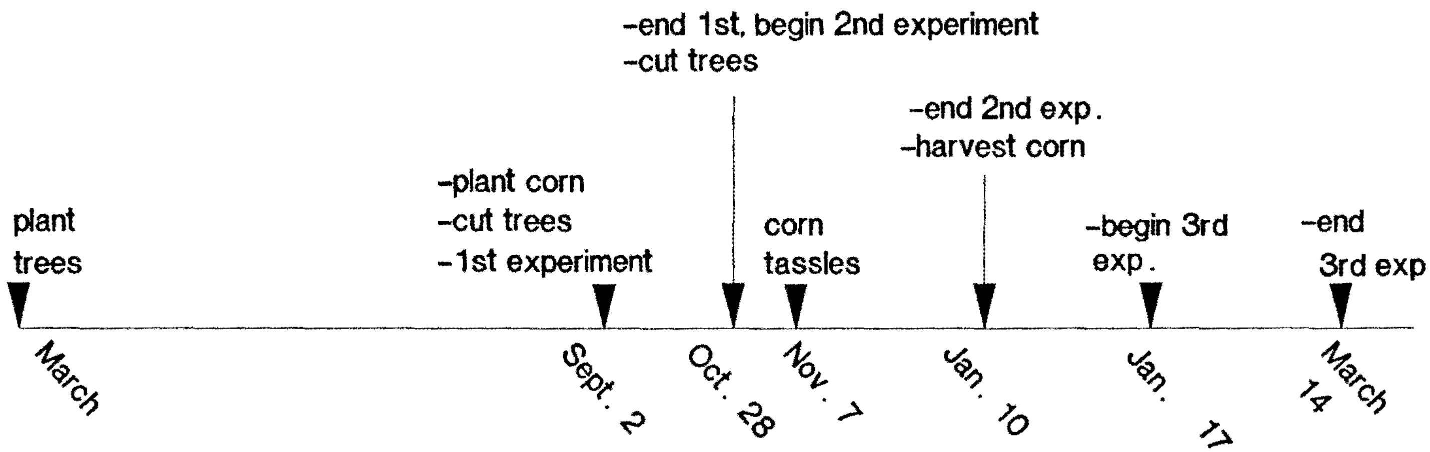

FIGURE 4.1 Schedule of the beginning and end of each experiment. Each experiment was 7.5 to 8 weeks with 3 or 5 harvest intervals (including initial and last harvest)

Soil nitrogen mineralization was measured by the buried bag technique (Eno, 1960; Smith et al., 1977; Westermann and Crothers, 1985) except that the soils were not sieved prior to incubation. Several soil cores were taken from a depth representing 0-25 cm, and placed into polyethylene bags that were then knotted. A portion of the samples was returned to the lab for immediate extraction while the other bags were returned to the sampling hole, covered with soil and harvested at later time periods. In the first experiment: four soil samples were extracted initially, four samples were harvested at 3 weeks and two soil samples were harvested at 4.5 and then at 8 weeks. Soils to be extracted were sieved at field-moisture through a 10 mesh sieve and then 15 g subsamples were extracted in 100 ml of 2N KCL for 30 minutes. After allowing the soil to settle overnight, a portion of the extract solution was filtered and frozen until subsequent analysis. For the second incubation experiment, 1 soil sample was extracted initially and 4 samples were collected at 4.5 and then at 7.5 weeks. The extraction procedure was the same except 17 g sub-samples were extracted in 0.5N K_2SO_4. For the third incubation period, one sample was extracted initially and then four samples were collected from the field at 4 and again at 8 weeks. The extraction procedure was the same as in the first incubation experiment.

ANALYSIS OF INORGANIC NITROGEN

Aliquots of the soil extracts were analyzed on a Technicon, AutoAnalyzer II for NH_4^+ (Gentry and Willis, 1988), NO_3^- and NO_2^- (Technicon, 1977).

Regression analysis was used to compare the nitrogen mineralization rates of the with and without residue treatments and the species treatments (Table 4.1 4.1). If the effects of time*GM were significant at the 0.05 level of probability, the Nmin rates were considered to be significantly different.

RESULTS

EFFECT OF RESIDUE AND NO-RESIDUE MANAGEMENT ON SOIL N MINERALIZATION

Only the third experiment resulted in treatment differences of the soil nitrogen mineralization rates. The soil with 11 and 19 week-old green manure residue mineralized nitrogen at a slower rate than the no residue treatment (Figure 4.2c). The largest difference in Nmin rate between the two treatments was for Gliricidia 1: 0.80 mg inorganic N/ kg soil/ week mineralized for the with-residue treatment and 1.64 mg inorganic N/ kg soil/ week for the no-residue treatment.

Nitrogen mineralization rates of the no residue and the no tree treatments were similar for each of the three experiments. In units of mg inorganic N/ kg soil/ week, they were: 1.03 for the no-residue and 1.61 for the no tree

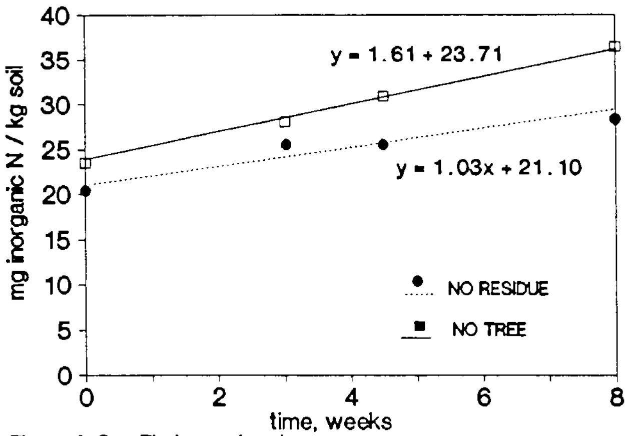

Figure 4.2a. First experiment

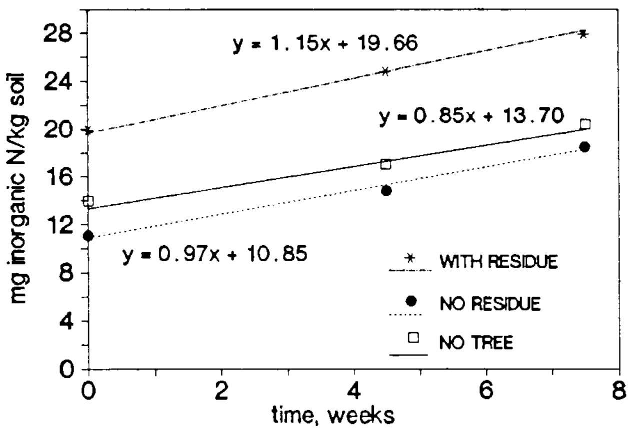

Figure 4.2b. Second experiment

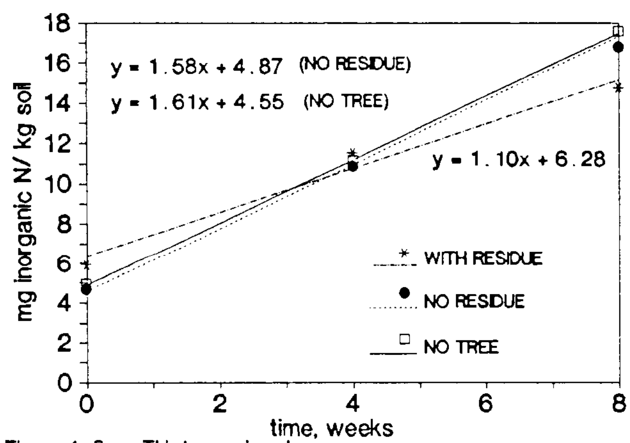

Figure 4.2c. Third experiment

FIGURE 4.2 Cumulative nitrogen mineralized in soil with residue, no residue and no residue, no tree ("no tree"). Individual points are means of all species. Each point is the mean of approximately: 10 values for the no tree treatment; 31 for the with residue and 20 for the no residue treatment.

in the first experiment; 0.97 for the no residue and 0.85 for the no tree in the second experiment; and 1.61 for the no tree and 1.58 for the no residue in the third experiment.

THE EFFECT OF DIFFERENT GREEN MANURE RESIDUES ON SOIL NITROGEN MINERALIZATION RATES

Comparisons of the nitrogen mineralization rates of soil with green manure residue from different species of leguminous trees found that there were no differences in the soil nitrogen mineralization rates. Also, removal of different green manures (no residue) did not effect different rates of soil nitrogen mineralization.
For the no residue treatment, this is illustrated by the tight clustering of data points at each sampling period (Figures 4.3a, 4.3b and 4.3c).

At certain time intervals of the second and third experiments, there were differences in the cumulative inorganic N mineralized for different green manure residue treatments (Figures 4.4a and 4.4b). What may appear as a decrease in the Nmin rate towards eight weeks for soil with Leucaena and Gliricidia residue (Figures 4.4a and 4.4b) is not significant. Due to water-logging of the samples, soil was not extracted and data was not available for Gliricidia 2 and Cassia reticulata in the second experiment.

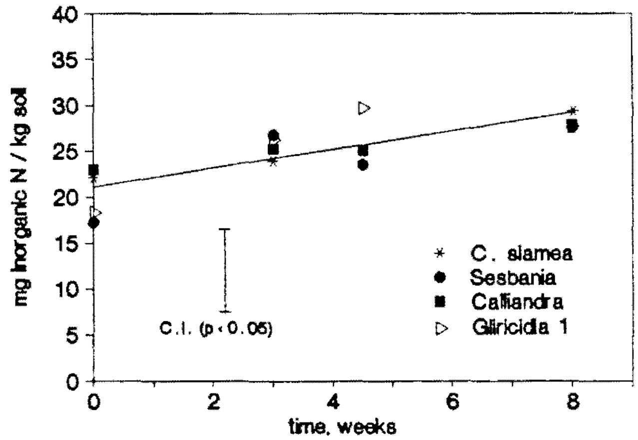

Figure 4.3a. First experiment

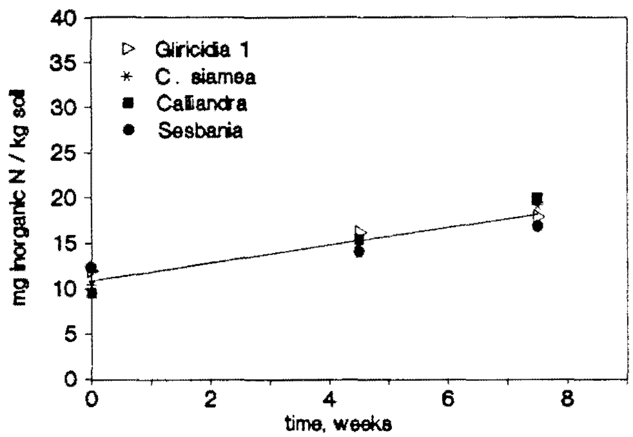

Figure 4.3b. Second experiment

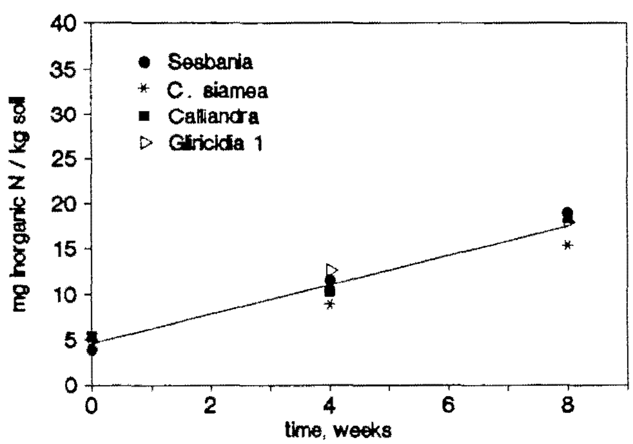

Figure 4.3c. Third experiment

FIGURE 4.3 Cumulative soil nitrogen mineralization when different green manures are removed and not applied (no residue)

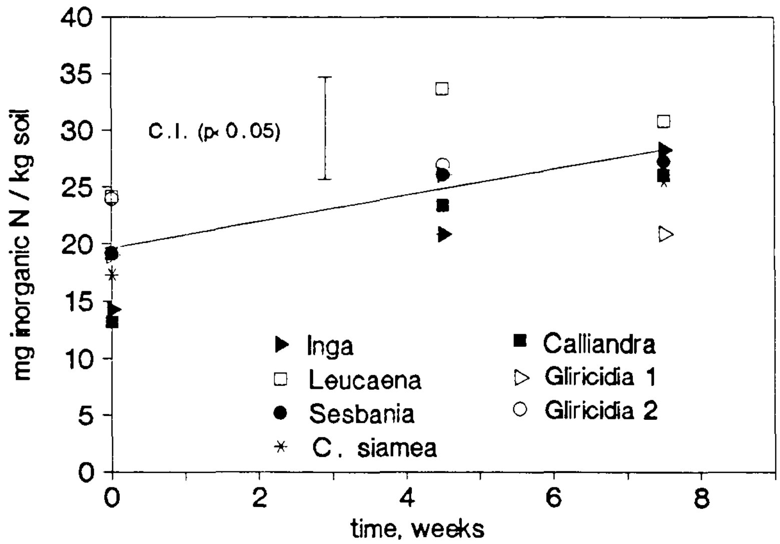

Figure 4.4a. Second experiment

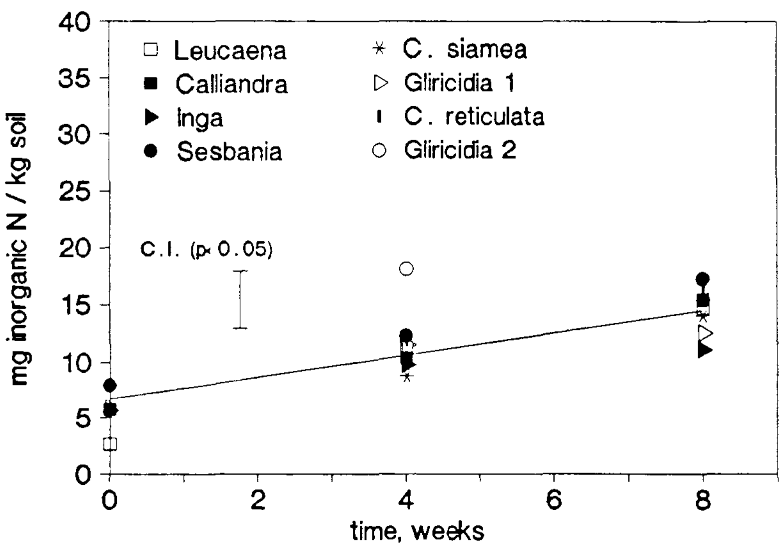

Figure 4.4b. Third experiment

FIGURE 4.4 Cumulative nitrogen mineralized in soil with different green manure residues.

DISCUSSION

THE EFFECT OF RESIDUE AND NO RESIDUE MANAGEMENT ON SOIL NITROGEN MINERALIZATION RATES

The lower rate of nitrogen mineralization in the residue versus the no residue treatment of the third experiment was not expected. The contrast of these results with those of Palm (1988) where the treatments were leaf residue and no residue, suggests that twigs may be effecting a reduced rate of nitrogen mineralization when residue is 11 and 19 weeks old. It should also be considered that the third experiment was started shortly after approximately 100 kg N/ ha of grain and stover had been harvested from the field. Therefore, the soil + residue mixture in these incubations probably contained mostly twig material and depleted available N. These conditions would be conducive to nitrogen immobilization which could have resulted in the reduced rate of nitrogen mineralization that was observed. Visual observations of the decomposition status of twigs in an earlier experiment (Chapter 3 of this thesis) indicated that Inga and Calliandra twigs had just begun to decompose at 12 weeks; and that Gliricidia twigs, although intact, showed the most signs of the onset of decomposition of any of the green manures at 12 weeks.

It is difficult to explain without further study why the nitrogen mineralization rate of soil with Gliricidia residue was so much slower than the nitrogen mineralization rate of soil that had not received Gliricidia green manure.

Further research should involve leaf and twig residue incubated separately as well as a mixture of the two. It would also be interesting to study how long before the residual effects of green manure residue effect higher rates of nitrogen mineralization than a no residue treatment. Low quality temperate forest litter has been shown to immobilize nitrogen for up to two years (McClaugherty *et al.*, 1985). The results from this study suggest that twigs play an important role in determining the residual effects of even such high quality green manures as *Leucaena* and *Gliricidia*. This indicates a need for better specifications when *Gliricidia* and perhaps other green manures are "advertised" as ideal green manures. Although it should be noted that fluctuating moisture conditions existing outside of the bags would accelerate nitrogen mineralization to some degree (Santana and Cabala-Rosand, 1982; Birch, 1958).

The similarity between the soil Nmin rates of the no tree and no residue treatments suggests that the relatively young leguminous trees (less than 1 year old) were not effecting the conversion rate of soil organic N to inorganic N. Therefore it appears that leguminous trees are not affecting the availability of soil inorganic nitrogen to an extent where microbial immobilization of soil organic N occurs. Vitousek and Denslow (1986) found unusually high nitrogen mineralization rates in their soils (using the buried bag method) and attributed this partly to an abundance of nodulated legumes growing in their study area.

THE EFFECT OF DIFFERENT GREEN MANURE RESIDUES ON SOIL NITROGEN MINERALIZATION RATES

Because of the range in quality of the green manure material (see Chapter 3 of this thesis), it was expected that residues of different green manures would effect differences in the nitrogen mineralization rate of soil N. However, the only observed differences between species were for the cumulative N mineralized at certain time periods. The cumulative N mineralized by Leucaena was different from at least one other species at each time period of the second experiment (Figure 4.4a).

The data for inorganic nitrogen accumulation at each time period was variable in all treatments of all three experiments. The coefficient of variation for the with residue treatment was the highest: 35-40% and was 24-33% for the no residue treatment. Fourteen percent of the bags in the second experiment had been punctured by twigs, gravel or insects. This degree of variability has been found in other studies that measure nitrogen mineralization of soil (Palm, 1988; Matson and Vitousek, 1981; Burke, 1989). The data variability is a reflection of the heterogeneous natural system that is being studied. Therefore, there is a tradeoff between mimicking natural conditions and detecting possible treatment differences. Repeating this portion of the study under more controlled conditions (such as a laboratory buried bag incubation) would help determine whether green manure residue from different species affects soil nitrogen mineralization rates differently.

CONCLUSIONS

This study showed that green manure residue can effect slower rates of soil nitrogen mineralization than when there is no residue present. Observations of the leaf and twig material at different time periods suggests that decomposing twigs could have immobilized N and contributed to these results. Fast-mineralizing green manures such as *Leucaena* and *Gliricidia* can contribute to higher levels of inorganic nitrogen at certain time periods but when compared to other green manure residues, these green manure residues were not found to effect higher rates of soil nitrogen mineralization. Effecting higher or lower rates of soil nitrogen mineralization would have important implications in determining the sustainability of an alley cropping practice managed with green manure applications.

TABLE 4.1 ANOVA's used for the three experiments to test the effect of cumulative soil nitrogen mineralized (complete ANOVA's are not listed due to the number of treatments and experiments)

<u>For testing Nmin rate differences between residue, no residue and no tree treatments (GM * time):</u>

rep
GM
sp
GM * sp
rep * trtmt
time
rep * time
GM * time
sp * time
GM * sp * time

<u>For testing Nmin rate differences between species of green manure within residue and no residue treatment (sp *time)</u>

rep
sp
rep * sp
time
rep * time
sp * time

<u>For testing Nmin rate differences between residue and no residue treatments for each species individually (GM*time):</u>

rep
GM
rep * GM
time
rep * time
GM * time

rep = replication
GM = residue, no residue, no tree
sp = tree species of green manure (eg. <u>Leucaena</u>)
trtmt = treatment (green manure and species)
time = incubation period in weeks

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